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(54) Title: USE OF FACTOR XIII FOR THE MANUFACTURE OF A MEDICAMENT FOR THE TREATMENT OF REPERFUSION INJURY AND MUCOSAL DAMAGE

(57) Abstract

Ischemic reperfusion injury, occurring spontaneously or resulting from ischemia induced to facilitate a surgical intervention, is reduced by administration of factor XIII in a biologically compatible vehicle. In addition, a factor XIII–containing pharmaceutical composition may be used to preserve the integrity of the mucosa or epithelium from damage caused by radiation or chemotherapeutic agents.

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USE OF FACTOR XIII FOR THE MANUFACTURE OF A MEDICAMENT FOR THE TREATMENT OF REPERFUSION INJURY AND MUCOSAL DAMAGE

TECHNICAL FIELD OF THE INVENTION

The present invention is directed to methods and compositions useful for reducing ischemic reperfusion injury or reducing the adverse effects of chemotherapy or radiation on the mucosa or epithelium. More particularly, the present invention is directed to methods and compositions useful for reducing necrotic tissue damage and/or vascular injury resulting from ischemic reperfusion or for reducing damage to mucosal integrity associated with chemotherapy or radiation treatment.

BACKGROUND OF THE INVENTION

Ischemia connotes the disruption of blood flow to normally perfused tissues. If an organ is ischemic for a sufficient time, cellular necrosis will occur. To prevent such necrosis, blood flow must be restored. However, significant injury to tissues and even death can occur as a result of reperfusion of previously ischemic tissue. Studies have shown that the ischemic insult is amplified by injury suffered upon reperfusion. Reperfusion following ischemia is important in a variety of clinical disorders, including stroke, myocardial infarction, organ transplantation and organ hypoperfusion.

Ischemic reperfusion injury is a complication associated with surgeries in which blood flow to tissue is interrupted and then resumed. Ischemic reperfusion injury can also arise when perfusion is interrupted by traumatic injury or endogenous blockage (*e.g.*, a partial or total occlusion of an artery, by deposition of materials as in

atherosclerosis or by formation of a blood clot) followed by restoration of blood flow to the tissues, either spontaneously or through intervention of a medical practitioner.

While some organ-specific differences exist, several generally applicable aspects of mechanism and features of ischemic reperfusion injury can be summarized. An important feature of early ischemia is the depletion of high energy chemical stores, such as ATP, with the accumulation of purine degradation products. Also, the enzyme xanthine dehydrogenase is converted to an oxidase. Subsequent reperfusion, wherein the oxygen supply to tissues is restored, permits xanthine oxidase to act on its purine substrate, resulting in the generation of oxidants. This process primarily occurs in organ parenchymal cells and in the endothelium.

Oxygen free radicals are generated upon reperfusion following ischemia and have been implicated in reperfusion injury. See Golino et al., Nature Medicine, 2(1): 35-40, 1996. Also, reperfusion injury is frequently associated with activation of the inflammatory system, an inflow of neutrophils into the previously ischemic tissue, and enhanced leukocyte-endothelial adhesiveness. Adherence and activation of neutrophils is generally considered to be a critical contributor to reperfusion injury, mediated by further release of oxidants and other injurious substances.

One of the consequences of the generation of oxygen free radicals and other oxidants and the neutrophil sequestration associated with ischemic reperfusion is vascular dysfunction resulting from changes in vascular tone and reactivity. Vascular tone is the major component of resistance to blood flow within the general circulation which, in turn, maintains appropriate arterial pressures at physiologic levels of cardiac output. Vascular reactivity is the responsiveness to neural, humoral and

vasomotor stimuli as well as to imposed physical forces. Appropriate responsiveness is necessary to maintain organ function and viability. Functional indications of reperfusion-induced endothelial dysfunction include increased vascular permeability to macromolecules, loss of endothelium-mediated vasodilation and release of endothelial-derived vasoconstrictors such as endothelin. Early histological evidence of endothelial damage includes swelling, bleb formation, cytoplasmic disorganization, junctional disruption and the like. Evidence exists that ischemic reperfusion injury extends to underlying layers of vessels, such as the basal lamina, and the smooth muscle of the vessel wall, with necrosis of the smooth muscle occurring upon more severe ischemic insults. Organ-specific consequences of ischemic reperfusion injury are also known in the art. See, for example, Conger et al., J. Investig. Med., 43(5): 431-42, 1995.

Blocking neutrophil adherence or aggregation with monoclonal antibodies has been shown to ameliorate reperfusion injury. See, for example, Winn et al., J. Clin. Invest., 92: 2042-7, 1993. Difficulties arise with regard to the use of such antibodies in the clinical setting, however. The neutrophil-endothelial cell interaction appears to be necessary to fight infection, which is also a complication of surgical procedures and often a causative agent in mortality arising from such procedures. Diminishing patient's ability to combat infection is therefore not preferred.

Also, chemotherapy and radiation treatments administered in the treatment of cancerous lesions have been shown to cause damage to the mucosa and epithelium. Damage to the mucosal barrier in the small intestine, for example, leads to egress of gastrointestinal flora into the blood stream. The acute injury to small intestinal mucosa associated with irradiation and cytotoxic chemotherapy appears to result from a high incidence of

apoptosis, particularly in crypt cells of the small intestine, migration of epithelial cells from the intestinal crypts toward the villi and a lack of mitotic counterbalance in such crypt cells. The incidence of apoptosis in normal small intestinal crypt cells is rare. Interleukin-11 has been shown in animal models to address such gastrointestinal injury. See, for example, Orazi et al., Laboratory Investigation 75(1): 33-42, 1996.

Similarly, ulcerative hemorrhagic cystitis of the bladder epithelium has been shown to result from cyclophosphamide chemotherapy. This condition has been prevented in animal models by administering keratinocyte growth factor (KGF). KGF is believed to fulfill this protective function by maintaining the integrity of bladder epithelium by urothelial cell proliferation-inducement or by cytoprotective mechanisms. See, for example, Ulich et al., Cancer Research 57: 472-5, 1997. Also, gastrointestinal mucositis is a known dose-limiting side effect of chemotherapy.

Factor XIII (also known as fibrin stabilizing factor, fibrinoligase, or plasma transglutaminase) is a plasma glycoprotein that circulates in blood as a zymogen (Mr=~320 kD) complexed with fibrinogen (Greenberg and Shuman, J. Biol. Chem., 257: 6096-6101, 1982). Plasma factor XIII zymogen is a tetramer consisting of two a subunits (Mr=~75 kD) and two b subunits (Mr=~80 kD) (Chung et al., J. Biol. Chem., 249: 940-950, 1974) having an overall structure designated as a2b2. The a subunit contains the catalytic site of the enzyme, while the b subunit is thought to stabilize the a subunit or to regulate the activation of factor XIII (Folk and Finlayson, Adv. Prot. Chem., 31: 1-133, 1977; Lorand et al., Biochem. Biophys. Res. Comm., 56: 914-922, 1974). The amino acid sequences of the a and b subunits are known (Ichinose et al., Biochemistry, 25: 6900-6906, 1986; Ichinose et al., Biochemistry, 25: 4633-4638, 1986).

Factor XIII occurs in placenta and platelets as an a2 homodimer.

In vivo, activated factor XIII (factor XIIIa) catalyzes cross-linking reactions between other protein molecules. During the final stages of blood coagulation, thrombin converts factor XIII zymogen to an intermediate form (a'2b2), which then dissociates in the presence of calcium ions to produce factor XIIIa, a homodimer of a' subunits. Placental factor XIII is activated upon cleavage by thrombin. Factor XIIIa is a transglutaminase that catalyzes the cross-linking of fibrin polymers through the formation of intermolecular n(c-glutamyl) lysine bonds, thereby increasing clot strength (Chen and Doolittle, Proc. Natl. Acad. Sci. USA, 66: 472-479, 1970; Pisano et al., Ann. N.Y. Acad. Sci., 202: 98-113, 1972). This cross-linking reaction requires the presence of calcium ions (Lorand et al., Prog. Hemost. Throm., 5: 245-290, 1980; Folk and Finlayson, Adv. Prot. Chem., 31: 1-133, 1977). Factor XIIIa also catalyzes the cross-linking of the c-chain of fibrin to a2-plasmin inhibitor and fibronectin, as well as the cross-linking of collagen and fibronectin, which may be related to wound healing (Sakata and Aoki, J. Clin. Invest., 65: 290-297, 1980; Mosher, J. Biol. Chem., 250: 6614-6621, 1975; Mosher and Chad, J. Clin. Invest., 64: 781-787, 1979; Folk and Finlayson, ibid.; Lorand et al., ibid.). The covalent incorporation of a2-plasmin inhibitor into the fibrin network may increase the resistance of the clot to lysis (Lorand et al., ibid.).

Factor XIII deficiency results in "delayed bleeding," but does not affect primary hemostasis (Lorand et al., ibid.) Current treatment practices for patients having factor XIII deficiencies generally involve replacement therapy with plasma or plasma derivatives, or with a crude placental factor XIII concentrate (Lorand et al., ibid.; Forbisch et al., Dtsch. med. Wochenschr., 97:

449-502, 1972; Kuratsuji et al., Haemostasis, 11: 229-234, 1982).

Factor XIII is also useful in treatment of patients with disorders in postoperative wound healing (Mishima et al., Chirurg, 55: 803-808, 1984; Baer et al., Zentrabl. Chir., 105: 642-651, 1980), scleroderma (Delbarre et al., Lancet, 2: 204, 1984; Guillevin et al., La Presse Medicale, 14: 2327-2329, 1985; Guillevin et al., Pharmatherapeutica, 4: 76-80, 1985; and Grivaux and Pieron, Rev. Pnemnol. Clin., 43: 102-103 1987), ulcerative colitis (Suzuki and Takamura, Throm. Haemostas., 58: 509, 1987), colitis pseudomembranous (Kuratsuji et al., Haemostasis, 11: 229-234, 1982) and as a prophylactic of rebleeding in patients with subarachnoid hemorrhage (Henze et al., Thromb. Haemostas., 58: 513, 1987). Furthermore, factor XIII has been used as a component of tissue adhesives (U.S. Patent Nos. 4,414,976; 4,453,939; 4,377,572; 4,362,567; 4,298,598; 4,265,233 and U.K. Patent No. 2 102 811 B).

Factor XIII has also been disclosed to be useful for reducing blood loss in patients undergoing surgery (International Patent Application No. PCT/US92/11241). Activity of factor XIII has been monitored in patients undergoing orthotopic liver transplantation. The interest in factor XIII in this context appears to result from the bleeding complications associated with the procedure (Himmelreich et al., Seminars in Thrombosis and Hemostasis, 19(3): 243-5, 1993. In addition, the use of factor XIII as an immunosuppressant has been postulated (U.S. Patent No. 5,404,615), although there is no consensus in the scientific community with regard to an immunosuppressive function of factor XIII. Also disclosed is the use of factor XIII in the treatment of blood coagulation disorders and metastatic tumors (PCT Patent Application No. PCT/EP90/01541).

There remains a need in the art for methods and compositions for reducing ischemic reperfusion injury and

preventing or ameliorating chemotherapy- or radiation-induced damage to mucosa or epithelium.

SUMMARY OF THE INVENTION

Within the present invention, factor XIII is used for the production of a pharmaceutical composition for the reduction of ischemic reperfusion injury or prevention or reduction of chemotherapy- or radiation treatment-induced mucosal or epithelial damage in a patient. Candidate patients for administration of a pharmaceutical composition for the reduction of ischemic reperfusion injury are those undergoing surgery to correct an ischemic state or undergoing surgery requiring induction of an ischemic state. Other candidate patients include those where ischemic reperfusion has occurred spontaneously, such as post-myocardial infarction or post-stroke. It will be recognized by one of ordinary skill in the art that the continued ischemia may necessitate surgical intervention. Candidate patients for administration of a pharmaceutical composition of the prevention or reduction of chemotherapy- or radiation treatment-induced mucosal or epithelial damage are those patients who have undergone or who are to undergo such procedures.

The present invention also provides methods for reducing ischemic reperfusion injury in a patient, wherein an effective amount of factor XIII in a biologically compatible vehicle is administered to the patient. Within one embodiment, the factor XIII composition is administered to the patient as a bolus injection, preferably within one day prior to a medical procedure. A series of daily bolus injections may also be employed. Additionally or alternatively, a factor XIII composition may be administered during and/or following the procedure. A continuous infusion mode of administration may be employed during the medical procedure. In an emergency

trauma-induced ischemia situation, such as an automobile accident wherein a patient suffers injuries resulting in disruption of blood flow to certain tissues, a bolus injection of a factor XIII composition may be administered by a paramedic or other medical practitioner at the scene of the accident. Other dosage forms useful in this context include gels, foams or bandages. Alternatively or additionally, a continuous infusion of the factor XIII composition may be employed en route from the scene of the traumatic injury to a medical facility.

The present invention also provides methods for reducing injury to mucosal or epithelial integrity resulting from radiation treatment or chemotherapy in a patient comprising administering to that patient an effective amount of factor XIII in a biologically compatible vehicle. In this embodiment, the biologically compatible vehicle may be designed for systemic administration, such as intravenous administration, or for local administration, such as an anal or vaginal suppository, an oral dosage form designed for factor XIII release in the small intestine rather than in the stomach. Chemotherapy and radiation treatments administered in the treatment of cancerous lesions have been shown to damage mucosa or epithelium. Sensitive mucosa include the mucosal barrier in the small intestine, the oral mucosa, anal mucosa, vaginal mucosa and the like. Sensitive epithelium include the bladder epithelium, kidney epithelium and the like. For these embodiments of the present invention, local administration of the pharmaceutical composition is preferred; however, systemic administration can also be used.

The factor XIII composition is generally administered at a dose sufficient to raise the patient's plasma factor XIII level to from about 150% to about 500% of the normal level thereof. A normal plasma level of factor XIII ranges from about 1 to about 2 mg/dl. A

preferred dose of factor XIII for use in the present invention ranges from 0.1-1.0 mg per kg of patient weight, preferably 0.15-0.4 mg per kg.

Factor XIII is a human protein. Thus, administration of factor XIII in a biologically compatible vehicle to human patients is not expected to elicit an immune response in those patients. Consequently, administration of a factor XIII composition in humans is expected to be safe.

These and other aspects of the invention will become evident upon reference to the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods and factor XIII compositions. These methods and compositions do not appear to negatively impact the function of the patient's immune system, thereby reducing the risk of infection and other adverse side effects. Also, the methods and compositions do not elicit a response from the patient's immune system, thereby enhancing the safety of the protocol and the efficacy of multiple doses.

In one embodiment of the present invention, factor XIII compositions are administered to patients to reduce ischemic reperfusion injury. This reduction is seen as a reduction in tissue damage or vascular injury during surgery or upon tissue trauma (ischemia), as a reduction in tissue damage or vascular injury following restoration of blood flow (reperfusion), as reduced recovery time, or a combination thereof. Administration before, upon or following reperfusion addresses ischemic reperfusion injury. These benefits are expected to contribute to a reduction in the cost of medical care.

Candidate patients for administration for treatment in accordance with the ischemic reperfusion embodiment of the present invention are those undergoing

surgery to correct an ischemic state or undergoing surgery requiring induction of an ischemic state to correct a medical problem. With respect to aspects of the present invention involving medical procedures, administration of factor XIII is unexpectedly useful in circumstances wherein significant bleeding complications are not anticipated. Such medical procedures include percutaneous transluminal coronary angioplasty (PTCA), bypass surgeries (e.g., cardiac or carotid), carotid endarterectomy and the like. Other candidate patients include those where ischemia and ischemic reperfusion have occurred spontaneously.

Correction of an ischemic state is required, for example, if the patient has suffered a traumatic injury resulting in severing of arteries or other disruption of blood flow to tissue. Examples of such traumatic injuries include knife wounds, gunshot wounds, many injuries caused by motor vehicle accidents and the like. Correction is also required when the patient is suffering from an endogenous blockage, such as from material deposition or other obstruction formation that interrupts blood flow. Examples of such conditions include atherosclerosis, blood clots, hyperreactive blood vessels and the like. A variety of procedures may be employed to remove the obstruction.

Induction of an ischemic state is required for many medical procedures. The more involved the procedure, the longer the ischemic state must be maintained. Generally, the length of the time of induced ischemia correlates with the severity of the ischemic reperfusion injury. Ischemia is induced, for example, in major thoracic and abdominal surgeries. Thoracic procedures include open-heart surgery and repeat cardiac surgery. Abdominal procedures include colonic resection and repair of liver or spleen trauma. Organ transplantation also requires induction of an ischemic state.

An endogenous ischemic state may also be disrupted spontaneously. This can occur, for example, in the context of myocardial infarction or stroke. In both cases, blood flow is blocked, creating an ischemic condition (e.g., an infarct, stroke or the like). Pressure builds as a result of the disruption in blood flow. Acute spontaneous release of that pressure constitutes ischemic reperfusion, typically generating additional tissue damage. The time frame for successful intervention for such conditions is quite short, generally from 1 to 12 hours with treatment preferably being administered as soon as possible. Consequently, treatments that are rapidly administrable and non-invasive, such as the methods and compositions of the present invention, are useful in addressing such conditions.

Other embodiments of the present invention involve methods for reducing injury to mucosal or epithelial integrity resulting from radiation treatment or chemotherapy in a patient comprising administering to that patient an effective amount of factor XIII in a biologically compatible vehicle. Chemotherapy and radiation treatment are currently employed in treatment of a variety of cancers, including solid tumor cancers, such as breast, colon, prostate, lung and the like, and other cancers such as leukemia, lymphoma and the like. Systemic administration of chemotherapeutic agents and radioisotopes expose non-cancerous tissue to those toxic agents. Even in treatment regimes involving targeted chemotherapeutic agents or radiation (e.g., via the use of antibodies to tumor-associated antigens), some non-target tissue is exposed. Tissue involved in elimination, such as the gastrointestinal tract, bladder and kidneys are especially susceptible to such exposure.

Chemotherapy and radiation treatments administered in the treatment of cancerous lesions have

been shown to damage mucosa or epithelium. Sensitive mucosa include the mucosal barrier in the small intestine, oral mucosa, anal mucosa, vaginal mucosa and the like. Sensitive epithelium include the bladder epithelium, kidney epithelium and the like. Thus, factor XIII compositions are useful for prevention or reduction of chemotherapy- and radiation-induced injury to mucosa and epithelium. Prophylactic administration is generally preferred.

Damage to the mucosal barrier in the small intestine, for example, leads to egress of gastrointestinal flora into the blood stream. The acute injury to small intestinal mucosa associated with irradiation and cytotoxic chemotherapy appears to result from a high incidence of apoptosis, particularly in crypt cells of the small intestine, migration of epithelial cells from the instestinal crypts toward the villi and a lack of mitotic counterbalance in such crypt cells. The incidence of apoptosis in normal small intestinal crypt cells is rare. Apoptosis can be monitored by the known nonisotopic ISEL technique, as described, for example, in Orazi et al., Laboratory Investigation 75(1): 33-42, 1996. Other assays known in the art to measure apoptosis may also be employed. Histologic and morphometric analysis of mice receiving cytoblative treatment may be employed to assess epithelial cell migration and crypt mitosis, also as described in Orazi et al. Alternatively or additionally, immunohistochemical analysis may be employed to assess the level of mitosis/proliferation. Antibodies directed to proliferating cell nuclear antigen (PCNA) are useful for this purpose. See Orazi et al. Alternatively, the TUNEL reagent may be employed in the study of apoptosis, as described, for example, by Gavrieli et al., J. Cell Biology 119(3): 493-501, 1993, and Negoescu et al., J. Histochemistry and Cytochemistry 44(9): 959-68, 1996.

With regard to epithelium, ulcerative hemorrhagic cystitis of the bladder epithelium, for example, has been shown to result from cyclophosphamide chemotherapy. Prevention of this condition has been achieved in animal models by administering an agent that is believed to maintain the integrity of bladder epithelium by urothelial cell proliferation-inducement or by cytoprotective mechanisms. Proliferation may be assessed by any known mechanism therefore, including immunohistochemical analysis using PCNA, as described, for example, in Ulich et al., Cancer Research 57: 472-5, 1997. Other cytoprotective mechanisms can be analyzed using assays known to those skilled in the art.

Within the present invention, an effective amount of factor XIII is combined with a biologically compatible vehicle and administered to a patient. Suitable vehicles include sterile, non-pyrogenic aqueous diluents, such as sterile water for injection, sterile buffered solutions or sterile saline. A preferred vehicle for the practice of the present invention includes glycine, EDTA and sucrose, as discussed in the Examples section hereof. The resulting composition is administered to the patient before and/or during and/or following reperfusion, chemotherapy or radiation treatment, preferably by intravenous injection, continuous infusion or local administration.

Within a preferred embodiment of the present invention, for use in medical procedures that are not generally characterized by significant bleeding complications, the factor XIII composition is administered as a bolus up to one week prior to the procedure, but preferably within one day prior thereto. Within another preferred embodiment, the factor XIII composition is administered as a daily bolus for 1 to about 5 days prior to the date of the procedure, with from 1 to about 3 days more preferred. In this daily bolus embodiment, an

additional bolus dose is preferably given to the patient within about 90 minutes of the commencement of the procedure, and more preferably within about 60 minutes thereof. Continuous infusion may also be used.

Alternatively or additionally, a dose of factor XIII composition may be administered to the patient during the procedure, particularly if unanticipated complications arise and the ischemic state must be maintained for a period of time greater than originally predicted. For such administrations, either a bolus dose or continuous infusion may be employed. Also alternatively or additionally, one or more doses of factor XIII composition may be administered following the procedure to ensure that the patient is protected from ischemic reperfusion injury. Post-procedure factor XIII composition administration may be conducted as a continuous infusion or in one or more bolus doses.

For emergency traumatic injuries or spontaneous ischemic reperfusions, one or more bolus injections or topical administration to the traumatized site are preferred for the initial administration of factor XIII composition. If topical administration directly to the afflicted area is practical based upon the nature of the injury/condition, such administration is generally preferred. Such topical administration can be achieved using factor XIII formulated as a gel or a foam or as an integral component of a bandage designed for use in the treatment of traumatic injuries. One of ordinary skill in the art is able to design such formulations. See, for example, Roberts & Travis, International Journal of Radiation Oncology Biology Physics 32(4): 1047-52, 1995 (wound dressing gel); Jones et al., J. Pharm. Pharmacol. Suppl. (United Kingdom) 43: 45, 1991 (polymeric films); Parodi et al., J. Dermatol. Treat. (United Kingdom) 1(6): 305-6, 1991 (medicated tape); Kuroyanagi et al., J. Burn Care Rehibil. (USA) 12(2) 106-115, 1991; Sawada et al.,

Br. J. Plast. Surg. (United Kingdom) 43(1): 83-7, 1990 (silicone gel sheet); and Attwood, Br. J. Plast. Surg. (United Kingdom) 42(4): 373-9, 1989 (calcium alginate dressing). When the patient requires further medical intervention, administration routes and protocols applicable to the procedure to be conducted will be employed.

Within a preferred embodiment of the present invention, for use to prevent or attenuate mucosal or epithelial damage caused by chemotherapy or radiation treatment, the factor XIII composition is administered as a daily bolus for 1 to about 5 days prior to the date of the therapy. Chemotherapy and radiation treatment protocols are often regimens of one to two week duration. Depending upon the chemotherapy or radiation treatment involved, a medical practitioner may administer additional bolus doses throughout the course of the protocol. A bolus dose of factor XIII composition may be systemically or locally administered. When local administration is possible, such administration is generally preferred. Oral administration can be employed to protect the oral mucosa, for example. Also, an oral dosage form designed to release factor XIII in the small intestine, rather than in the stomach, can be employed to protect the intestinal mucosa. See, for example, the discussion of enteric-coated tablets set forth in Ozturk et al., Pharm. Res. (United States) 5(9): 550-65, 1988, and other works on the subject, such as Nishihata et al., J. Pharm. Pharmacol. (United Kingdom) 45(11):947-50, 1993, Davis, J. Contol. Release (Netherlands) 2: 27-38, 1985, Lavelle et al., Advanced Drug Delivery Reviews (Netherlands) 18(1): 5-22, 1995, and Wilding et al., Pharmocol. Ther. (United Kingdom) 62(1-2): 97-124, 1994. In addition, anal or vaginal suppositories may be employed to protect mucosa located in those cavities. Continuous infusion may also be used.

Administration may be conducted in any manner allowing the factor XIII composition access to the tissues of interest. Preferably, the factor XIII composition is administered via intravenous, intraarterial, oral, mucosal (via a suppository or like administration mode) or like routes of administration.

Factor XIII (also known as "fibrinoligase" [Lorand et al., Prog. Hemost. Thromb., 5: 245-290, 1980] and "fibrin stabilizing factor" [Curtis and Lorand, Methods Enzymol., 45: 177-191, 1976]) is characterized by its ability, when activated, to form intermolecular c-glutamyl-e-lysine cross links between side chains of fibrin molecules and between other substrates. The enzyme exists in plasma as a tetrameric zymogen of two a subunits and two b subunits (designated a2b2), but is found in other tissue as an a2 dimer. Either of these zymogen forms may be used within the present invention, as well as genetically engineered variants of factor XIII that retain its characteristic cross-linking activity. It has been unexpectedly found by the present inventors that factor XIII is effective to reduce ischemic reperfusion injury or to preserve musosal or epithelial integrity from the ravages of chemotherapy or radiation treatment.

Within one embodiment of the present invention an "effective amount" of factor XIII is defined as that amount sufficient to reduce ischemic reperfusion injury or the associated hospital stay of the patient. Preferably, the factor XIII composition reduces tissue damage by at least 15% or reduces the hospital stay of the patient by at least one day. The extent of tissue damage resulting from ischemic reperfusion injury will vary somewhat depending on the tissue involved. Medical practitioners are able to both predict the timing of tissue damage and determine the extent of that tissue damage. Models of ischemic reperfusion injury in different organs are known in the art and are helpful in this regard. Alternative

measures of function may also be used to determine efficacy, such as performance in a treadmill test, dye injection to measure cardiac output and the like. Thus, one of ordinary skill in the art is capable of determining an effective amount of factor XIII composition.

The amount of factor XIII in the composition to be administered will be sufficient to provide a supranormal plasma level of factor XIII upon completion of the administration protocol. A normal plasma level of factor XIII ranges from about 1 to about 2 mg/dl. A preferred embodiment of the present invention involves increasing the plasma level of factor XIII to at least 100% of normal, with an increase of from about 150% to about 500% more preferred. An effective amount of factor XIII will generally be in the range of about 0.1 to 1.0 mg per kg of patient weight, i.e. a dose of about 10 mg to about 70 mg for a 70 kg patient. Doses in the range of about 0.15 mg to 0.4 mg per kg of patient weight are particularly preferred. The actual amount of factor XIII administered will depend in part on such factors as the nature of the medical procedure, the ischemic/reperfusion injury involved, and the patient's medical status. For example, traumatic injury will generally necessitate higher doses than surgery preparation.

In embodiments of the present invention wherein the factor XIII composition is administered to prevent or attenuate mucosal or epithelial damage caused by radiation or chemotherapy, an "effective amount" of factor XIII is defined as that amount sufficient to reduce mucosal or epithelial damage caused by chemotherapy or radiation treatment or the associated hospital stay of the patient. Since disruptions in mucosal or epithelial integrity often leads to infection, reduction of mucosal and epithelial damage may be assessed indirectly by monitoring the incidence or severity of infection, body temperature or the like. Doses employed for these purposes will

generally be similar to those mg/kg body weight doses discussed above with regard to ischemic reperfusion injury.

In the experiments included in the Examples section of this patent application, a rat model of gut ischemia was employed to evaluate a factor XIII composition for reduction of ischemic reperfusion injury. Visual inspection of the rats exposed to induced gut ischemia generally revealed pronounced tissue damage in the vehicle-treated rats, with either less severe or imperceptible tissue damage in the factor XIII composition-treated rats. Histology confirmed this result, revealing that rats receiving the factor XIII composition generally suffered less tissue damage than the control rats. Moreover, following surgery and prior to sacrifice, the factor XIII composition-treated rats appeared to be more active and in less pain than the vehicle-treated rats. In addition, a trend toward lowered myeloperoxidase (MPO) and elevated maltase levels was observed in factor XIII-treated animals. Generally, reduced MPO indicates reduced white blood cell accumulation and/or activity. Much of the damage attributable to ischemic reperfusion injury involves accumulation of such cells. Typically, elevated maltase indicates preservation of mucosal integrity.

Factor XIII for use within the present invention may be prepared from plasma according to known methods, such as those disclosed by Cooke and Holbrook (Biochem. J., 141: 79-84, 1974) and Curtis and Lorand (Methods Enzymol., 45: 177-191, 1976), incorporated herein by reference. The a2 dimer form of factor XIII may be prepared from placenta as disclosed in U.S. Patents 3,904,751; 3,931,399; 4,597,899 and 4,285,933, incorporated herein by reference. It is preferred, however, to use recombinant factor XIII so as to avoid the

use of blood- or tissue-derived products that carry a risk of disease transmission.

Methods for preparing recombinant factor XIII are known in the art. See, for example, Davie et al., EP 268,772 and Grundmann et al., AU-A-69896/87, which are incorporated herein by reference in their entirety. Within a preferred embodiment, the factor XIII a2 dimer is prepared cytoplasmically in the yeast Saccharomyces cerevisiae as disclosed in copending United States Patent Application Serial No. 08/333,236 and PCT Application US92/06629, incorporated herein by reference in their entirety. The cells are harvested and lysed, and a cleared lysate is prepared. The lysate is fractionated by anion exchange chromatography at neutral to slightly alkaline pH using a column of derivatized agarose, such as DEAE Fast-Flow SepharoseTM (Pharmacia) or the like. Factor XIII is then precipitated from the column eluate by concentrating the eluate and adjusting the pH to 5.2-5.5, such as by diafiltration against ammonium succinate buffer. The precipitate is then dissolved and further purified using conventional chromatographic techniques, such as gel filtration and hydrophobic interaction chromatography.

As will be appreciated by those skilled in the art, it is preferred to use a factor XIII protein syngeneic with the patient in order to reduce the risk of inducing an immune response. Preparation and characterization of non-human factor XIII has been disclosed by Nakamura et al. (J. Biochem., 78: 1247-1266, 1975). The present invention encompasses the use of such factor XIII proteins within veterinary procedures, such as strangulated gut and the like.

The following examples are offered by way of illustration, not limitation.

Example 1

Male Sprague Dawley rats weighing between 350 and 400 grams were fasted for 18 hours, and anesthetized with sodium pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, Illinois). The right jugular vein and left carotid artery of each rat were catheterized with microbore tygon tubing (0.016 in. I.D. X 0.031 in. O.D.; Norton Co., Akron, Ohio) for the ischemia reperfusion studies. The catheters were filled with 30% PVP (Polyvinylpyrrolidon K 30; Fluka Chemical Corp., Ronkonkoma, New York) and 500 U/ml of heparin (Sigma Chemical Co., St. Louis, Missouri) to maintain patency. The rats were placed in restrainer cages and received administrations of the test composition or vehicle as described below.

The rats were weighed and rat #1 (0.371 kg) was injected with vehicle (0.1 mM EDTA, 10 mM glycine, 2% sucrose) and rat #2 (0.386 kg) was injected with the factor XIII composition (same as for vehicle except that sufficient factor XIII was added for a 1 mg/kg dose). Both compositions were in powder form, and reconstituted with 0.9% sterile saline. The vehicle or factor XIII composition for each rat was 1 mg/kg/day, 0.5 ml intravenous bolus, for 4 days. The rats were fasted for 18 hours prior to the fourth injection, anesthetized with Nembutol® (50 mg/ml, 0.090 ml/100 gms), injected with vehicle or factor XIII composition. Thirty minutes following this fourth injection, the superior mesenteric artery of each rat was clamped for two hours, using a micro aneurysm clip (100 grams, pressure, Roboz Surgical, Rockville, Maryland). The clamp was then released, and the rats were observed for approximately sixty minutes before the abdominal cavity was sutured shut. The rats were placed in a cage onto heating pads to recover from surgery. Following a recovery period of about 5 hours, the rats were placed in individual cages with access to

chow and water for observation. Following the observation period of about 24 hours, the rats were anesthetized with Nembutol®, and the abdominal incision was reopened. The intestinal segment of the rat abdomen was excised and placed in 10% NBF (neutral buffered formalin; SurgiPath Medical Indust., Richmond, Illinois) for histology. Photographs were taken of the excised gut of both animals. Histology was performed on the excised gut by standard hemotoxylin and eosin staining on a longitudinal section of the jejunum, and histology slide photographs were taken.

During the recovery and observation period, rat #1 (vehicle) looked less healthy than rat #2. Rat #1 showed signs of pilo erection with less body movement in comparison to rat #2. Gross histology of rat #1 showed small intestine and stomach inflated with bloody fluid and capillary damage along the gut wall. Rat #2 exhibited some damage to the gut, but the degree of damage was far less than that experienced by rat #1. This gross histology indicates that a positive or preventative effect on gut ischemia was exhibited by rat #2 (factor XIII) and that rat #1 (vehicle) showed apparent ischemia reperfusion injury.

A more detailed histological evaluation of the results also verified a positive or preventative effect of factor XIII treatment. The histology score for the factor XIII composition-treated rat was 0.13, while the score for the vehicle-treated rat was 0.75, indicated that the tissue sample from the vehicle-treated rat exhibited greater ischemia reperfusion-induced injury than that of the factor XIII composition-treated rat.

<u>Example 2</u>

Male Sprague Dawley rats weighing between 225 and 400 grams were fasted for 18 hours, anesthetized with Nembutal® and a silicone catheter (0.25 in. I.D. X 0.47

in. O.D.; Dow Corning Company, Midland, Michigan) was surgically placed in the right jugular vein for the ischemia reperfusion studies. The catheters were filled with 30% PVP (Polyvinylpyrrolidon K 30; Fluka Chemical Corp., Ronkonkoma, New York) and 500 U/ml of heparin (Sigma Chemical Co., St. Louis, Missouri) to maintain patency. The rats were placed in restrainer cages and received administrations of the test composition or vehicle as described below.

Rats #1, #4 and #5 (weighing 0.343 kg, 0.329 kg and 0.294 kg, respectively) were injected with the factor XIII composition described in Example 1 above. Rats #2 and #3 (weighing 0.293 kg and 0.328 kg, respectively) were injected with the vehicle described in Example 1 above. The vehicle or factor XIII composition for each rat was 1 mg/kg/day, 0.5 ml intravenous bolus, for 4 days. The rats were fasted for 18 hours prior to the fourth injection, anesthetized with Nembutol® 50 mg/ml (0.090 ml/100 gms), injected with vehicle or factor XIII composition. Thirty minutes following this fourth injection, the superior mesenteric artery of each rat was clamped for two hours, using a Micro-serrefines vascular clamp (100 grams, pressure; Fine Science Tools, Inc. Foster City, California). The clamp was then released, and photographs were taken of the small intestine of each rat 5-10 minutes thereafter. Upon visual inspection, rats that received the factor XIII composition looked healthier than the vehicle-treated rats.

The rats were observed for approximately sixty minutes before the abdominal cavity was sutured shut. Rats #3, #4 and #5 died sixty minutes after clamp release, most likely from the combination of additional anesthetic administered just prior to closing the abdominal wall and gut reperfusion of cytokines. Rats #1 and #2 did not require any additional anesthetic prior to closing. The rats were placed in a cage onto heating pads to recover

from surgery. Following a recovery period of about 5 hours, the rats were placed in individual cages with access to chow and water for observation. Following the observation period of about 24 hours, the rats were anesthetized with Nembutol®, and the abdominal incision was reopened. A small intestinal segment of the rat abdomen and additional tissue (lung, kidney, cecum and liver) were excised and placed in 10% NBF for histology using standard hematoxylin and eosin staining. Photographs were taken of the excised gut of both animals. Histology was performed on the excised gut, stomach, cecum, kidney and lung tissue.

During the recovery and observation period, rat #2 (vehicle) looked sick and was very lethargic (remained in one area of the cage even when the cage was shaken), In addition, rat #2 was cool to the touch. In contrast, rat #1 (factor XIII) looked healthier than rat #2, was alert and moving about in his cage. Moreover, rat #1 was warm to the touch. Gross histology of rat #2 (vehicle) showed that the gut was full of blood from stomach to colon. In contrast, the gut of rat #1 (factor XIII) looked virtually normal. This gross histology indicates that a positive or preventative effect on gut ischemia was exhibited by rat #1 (factor XIII) and that rat #2 (vehicle) showed apparent ischemia reperfusion injury.

A more detailed histological evaluation of the results also verified a positive or preventative effect of factor XIII treatment. The histology score for the factor XIII composition-treated rat was 0.25, while the score for the vehicle-treated rat was 0.75, indicated that the tissue sample from the vehicle-treated rat exhibited greater ischemia reperfusion-induced injury than that of the factor XIII composition-treated rat.

Example 3

This example summarizes results from two animal experiments, differing only slightly as described herein.

Rats underwent three training sessions with regard to sitting quietly in restraining cages. Next, the rats underwent a survival surgery, during which jugular vein catheters were implanted. For the survival surgery, rats were anesthetized with sodium pentobarbitol (Nembutol®, 50 mg/ml, 0.1 ml/100 g), and a silastic catheter (0.25 in. I.D. X 0.47 in O.D.; Dow Corning Company, Midland, Michigan) was implanted in the right jugular vein. The catheters were filled with 30% PVP (Polyvinylpyrrolidon K 30; Fluka Chemical Corp., Ronkonkoma, New York) and 500 U/ml of heparin (Sigma Chemical Co., St. Louis, Missouri) to maintain patency. The rats were placed in restrainer cages and received administrations of the test composition or vehicle as described below.

The rats were allowed to recover for 48 hours prior to a 4 day single intravenous bolus injection (0.5 ml) per day of either vehicle or factor XIII composition as described in Example 1. These injections were given with the scientist blinded to the treatment given. The injections were given to rats placed in restraining cages. The rats were fasted for 18 hours prior to the fourth injection. The rats were anesthetized via an intraperitoneal injection of an anesthetic cocktail mixture (5 ml ketamine HCl in injectable form; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa), 1.6 ml Rompun® (Phoenix Pharmaceutical, St. Joseph, Missouri) and 26,4 ml of phosphate buffered saline, 0.4 ml/100 gm body weight) and given an analgesic (buprenorphine hydrochloride, 0.1 ml/rat given i.m.; Reckitt and Colman Pharmaceuticals, Inc., Richmond, Virginia). Then the fourth injection was given.

Blood samples were taken via the jugular vein catheter or by retroorbital sinus bleeding just prior to clamping the superior mesenteric artery as well as at the end of the study for animals in one but not both studies. Blood was drawn to measure complete blood counts (0.5 ml in EDTA tubes) and for flow cytometry experiments (1.0 ml + 30 µl heparin at 1000 U/ml).

Thirty minutes after the fourth injection, the abdomen of each rat was opened with a small incision, and the superior mesenteric artery was isolated and clamped for one hour, using an arterial atraumatic clamp of 100 g pressure (Fine Science Tools, Inc., Foster City, California). The abdomen was loosely sutured closed during the clamping period, reopened for removal of the clamp and again loosely sutured closed. The rats were placed into holding cages resting on a 37°C heating pad for a two hour reperfusion period. Following the reperfusion period, the rats were sacrificed and two 5 cm jejunal intestinal segments were excised, 25-30 cm and 30-35 cm upstream from the ilieocecal junctions. The first 5 cm segment, 25-30 cm, was flash frozen in liquid nitrogen and placed on dry ice for later analysis for myeloperoxidase (MPO) and maltase activities, and for determination of protein concentrations. The second 5 cm segment, 30-35 cm, was placed into 10% NBF for histological evaluation using standard hematoxylin and eosin staining.

MPO is a measure of the amount of neutrophil infiltration into the tissue, while maltase activity is a measure of the integrity of the intestinal mucosa. Ischemic reperfusion injury is associated with increased levels of MPO and reduced levels of maltase activity. Consequently, amelioration of ischemic reperfusion injury is expected to result in reduced MPO and increased maltase activity.

In one study, rats receiving a 4 day pretreatment with the factor XIII composition exhibited

reduced MPO activity, reduced degree of histopathology and increased maltase activity. Five rats were treated with vehicle and five rats were treated with the factor XIII composition in this study.

In another study, eight rats were included in each treatment group. Again, rats receiving the factor XIII composition exhibited reduced MPO activity and reduced degree of histopathology. No effect on maltase activity was observed in this study. Both studies revealed a trend for factor XIII to reduce intestinal ischemic reperfusion injury in a rat model characterized by a one hour clamp time and a two hour reperfusion time.

Other animal models of ischemic reperfusion injury are known and used by persons skilled in the art. For example, Golino et al., Nature Medicine, 2(1): 35-40, 1996, describe a myocardial model of ischemic reperfusion injury employing New Zealand white rabbits. New Zealand white rabbits have also been employed in (1) an ischemic reperfusion model of the central vein in the ear and (2) a atherosclerotic femoral artery injury model in which blood flow is reinstated by balloon angioplasty. See, for example, , Winn et al., J. Clin. Invest., 92: 2042-7, 1993, and Jang et al., Circulation, 92(10): 3041-50, 1995. These animal models are useful to establish the efficacy of factor XIII-containing compositions for the reduction of ischemic reperfusion injury in additional organs.

Although certain embodiments of the invention have been described in detail for purposes of illustration, it will be readily apparent to those skilled in the art that the methods and formulations described herein may be modified without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

We claim:

1. A method of reducing ischemic reperfusion injury in a patient at risk of or suffering from spontaneous ischemic reperfusion or trauma induced-ischemia comprising administering to said patient an effective amount of factor XIII in a biologically compatible vehicle.

2. A method of reducing ischemic reperfusion injury in a patient at risk of such injury undergoing a medical procedure which is not expected to be associated with problematic postoperative bleeding, which method comprises administering to said patient an effective amount of factor XIII in a biologically compatible vehicle.

3. A method of reducing injury to mucosal or epithelial integrity resulting from radiation treatment or chemotherapy in a patient comprising administering to said patient an effective amount of factor XIII in a biologically compatible vehicle.

4. The use of factor XIII for the treatment of ischemic reperfusion injury in a patient at risk of or suffering from spontaneous ischemic reperfusion or trauma induced-ischemia.

5. The use of factor XIII for the treatment of ischemic reperfusion injury in a patient at risk of such injury undergoing a medical procedure which is not expected to be associated with problematic postoperative bleeding.

6. The use of factor XIII for the treatment of injury to mucosal or epithelial integrity resulting from radiation treatment or chemotherapy in a patient.

7. The use of factor XIII for the manufacture of a pharmaceutical composition for the treatment of ischemic reperfusion injury in a patient at risk of or suffering from spontaneous ischemic reperfusion or trauma induced-ischemia.

8. The use of factor XIII for the manufacture of a pharmaceutical composition for the treatment of ischemic reperfusion injury in a patient at risk of such injury undergoing a medical procedure which is not expected to be associated with problematic postoperative bleeding.

9. The use of factor XIII for the manufacture of a pharmaceutical composition for the treatment of injury to mucosal or epithelial integrity resulting from radiation treatment or chemotherapy in a patient.

INTERNATIONAL SEARCH REPORT

Intern. ⁚ .nal Application No
PCT/US 98/09742

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/45

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	DE 38 29 524 A (BEHRINGWERKE AG) 1 March 1990 see page 2, line 58 - line 63 see page 3, line 1 - line 6; claims 1-6	1-9
X	US 5 464 615 A (THOMAS STIEF ET AL.) 7 November 1995 see column 2, line 50 - line 57	1,2,4,5, 7,8
X	Medline Database, Accession no. 94210720 & Rinsho Ketsuki. Japanese Journal of Clinical Hematology, vol. 35, no. 3, pages 279-85 XP002076601 see abstract	3,6,9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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